Occupational asthma caused by IgE-mediated sensitization to multiple woods

Wood is a natural material that is able to trigger rhinitis and asthma in exposed subjects in occupational settings. This has been described with both soft and hard woods.[1,2] Involvement of both low- and high-molecular-weight allergens has been reported, and the relevance of these is related with the wood type.[1] There are cases where protein may be the responsible allergen. Cross-reactivity between obeche and ramin woods[3] and between obeche and latex[4] has been shown. However, to the best of our knowledge, this is the first report of a multiple IgE-mediated sensitization to different woods that caused occupational respiratory symptoms in the same worker.

A 40-year-old man who worked in carpentry for 12 years was referred for developing symptoms of nasal pruritus, blockage, sneezing, and runny nose while cutting and sanding wood. Symptoms started 10 years ago and were mild. The symptoms worsened in the last 2 years, with the patient developing episodes of dry cough, chest tightness, intense dyspnea, and wheezing 20 to 30 minutes after starting his working shift and needing treatment at the emergency department because of severe bronchospasm. The patient worked in a wood factory where, despite the ventilation, substantial amounts of wood dust were visible in the air. He related his symptoms with exposure to obeche (*Triplochiton scleroxylon*, also known as African maple or samba wood), iroko (*Milicia excelsa*), cerejeira (*Amburana cearensis*), oak (*Quercus* sp), and pine (*Pinus* sp) wood that were used regularly in his workplace. The physical examination of the patient was normal, and so were the hemogram and basic blood biochemistry. Total IgE was 275 IU/mL, and chest x-ray showed no abnormalities. Spirometry showed FEV_1 87%, FEV_1/FVC 85%, and mild obstruction of midlung volume flow (forced expiratory flow at 25%-75% of forced vital capacity 62%) with the negative bronchodilator test (<12% change in FEV_1). Skin prick tests to a battery of common aeroallergens showed positive results to grass pollen and dust mites. To further investigate a possible sensitization to wood, in-house extracts were made with the 5 aforementioned woods provided by the patient as described[5] (see this article's Online Repository

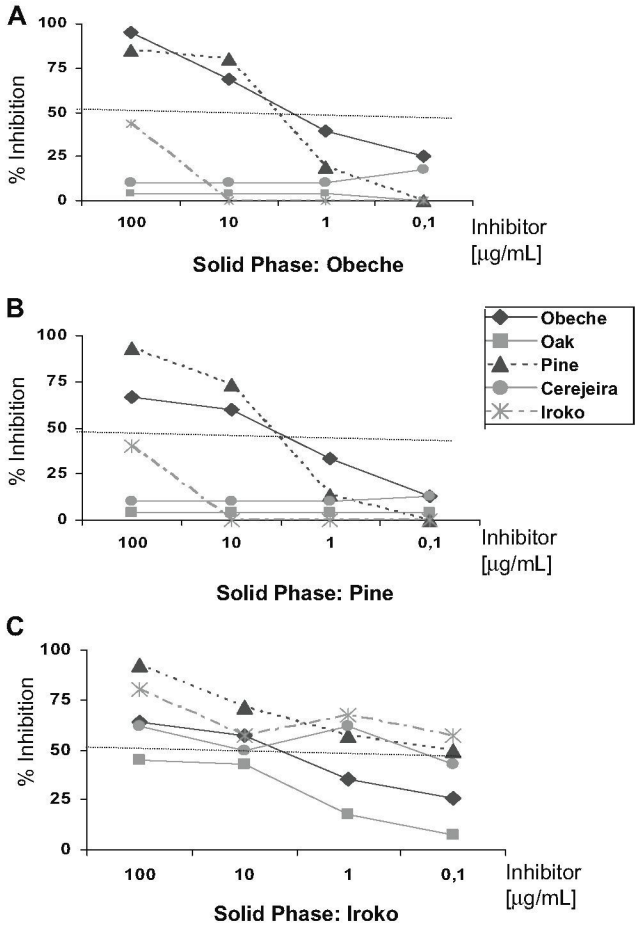

FIG 1. ELISA cross-inhibition assay with each extract as solid phase (30 μg/mL) and the other woods used as inhibitors in liquid phase (100, 10, 1, and 0.1 μg/mL) incubated with patient's serum. **A,** Solid phase: obeche; **B,** solid phase: pine tree; **C,** solid phase: iroko.

at www.jacionline.org). Skin prick tests with the extracts (1 mg/mL) were positive to obeche (8 mm), iroko (10 mm), cerejeira (3.5 mm), and pine (10 mm) and negative to oak. Specific IgE to these woods was measured by RAST as described,[6] showing positive results to obeche (38%) and pine tree (6.9%), a weak positive response to iroko (1.5%, positive cutoff point 0.5%), and negative results to cerejeira and oak. The same results were obtained by performing the ELISA test.[7] Skin prick testing, RAST, and ELISA assays were performed in 10 controls all of whom had negative responses. Written informed consent was obtained from all participants, and the ethical committee of our institution approved the study. Methacholine challenge was performed at baseline, yielding a PC_{20} value of 0.96 mg/mL (normal value > 16.0 mg/mL), and induced sputum before and after challenge. Specific inhalation challenges were performed by using the same extracts (1 mg/mL) in aqueous form with serial dilutions and using a DeVilbiss device (DeVilbiss, Somerset, Pa). Challenges were performed on different days for each wood and separated 4 weeks after a control day. Bronchial challenges were positive (≥20% fall in FEV_1) to obeche (1:1000), iroko (1:100), cerejeira (1:10), and pine (1:100) extracts and negative to oak. All responses were immediate (30-45 minutes) except for obeche wood (90 minutes), which caused a significant proportional increase in sputum eosinophils (4.5% vs 16.9%) and a decrease in neutrophils (89.6% vs 59.5%) after challenge. Since a multiple sensitization was demonstrated, the possible cross-reactivity between woods was further analyzed by ELISA cross-inhibition assays (see this article's Online Repository at www.jacionline.org). As shown in Fig 1, *A*, pine showed a strong inhibition against obeche (85%), iroko showed a weak inhibition (below 50%), and cerejeira or oak extracts showed no inhibition. A similar pattern was observed when pine was used as solid phase (Fig 1, *B*), where obeche inhibits pine by almost 70%. Finally, when iroko was used as solid phase, we observed that once again pine acts as a potent inhibitor (90% inhibition), followed by a more modest although significant inhibition by obeche (64%) and cerejeira (62%). SDS-PAGE and Western blot assays with obeche and pine extracts (see Fig E1 in this article's Online Repository at www.jacionline.org) showed bands of approximately 14, 28, 38, and 58 kDa in the obeche extract but no apparent bands in the pine extract. As described in other woods such as red cedar or iroko,[8] a low-molecular-weight substance (abietic acid) could be the causative agent in asthma induced by pine. Our pine extract was then tested by using gas chromatography combined with mass spectrometry (see this article's Online Repository at www.jacionline.org), detecting abietic acid (23%) and dehydroabietic acid (51%) as principal low-molecular-weight compounds (Fig 2). An ELISA inhibition immunoassay did not show any significant inhibition of patient's IgE binding by using free abietic acid as a liquid phase inhibitor (up to 1000 μg/mL) and the pine extract as solid phase (30 μg/mL) (data not shown).

We report a case of a multiple sensitization to different woods demonstrated by *in vivo* and *in vitro* tests. Cross-reactivity among different woods was assessed by using ELISA cross-inhibition assays. Surprisingly, pine extract was a strong inhibitor of 2 tropical woods such as obeche (genus *Sterculia*, family Sterculiaceae) and iroko (genus *Milicia*, family Moreaceae), which are unrelated to pine (genus *Pinus*, family Pineaceae). Information about wood allergens is scarce with few exceptions,[1,2] and so the nature of the allergen(s) responsible for the multiple sensitizations in this patient is still unknown; although the presence of low-molecular-weight compounds such as abietic acid and dehydroabietic acid was demonstrated in the extract by using gas chromatography combined with mass spectrometry, no IgE-binding activity was detected. Further studies are necessary to characterize wood antigens and develop standardized extracts to improve the diagnosis of occupational asthma caused by wood dust.

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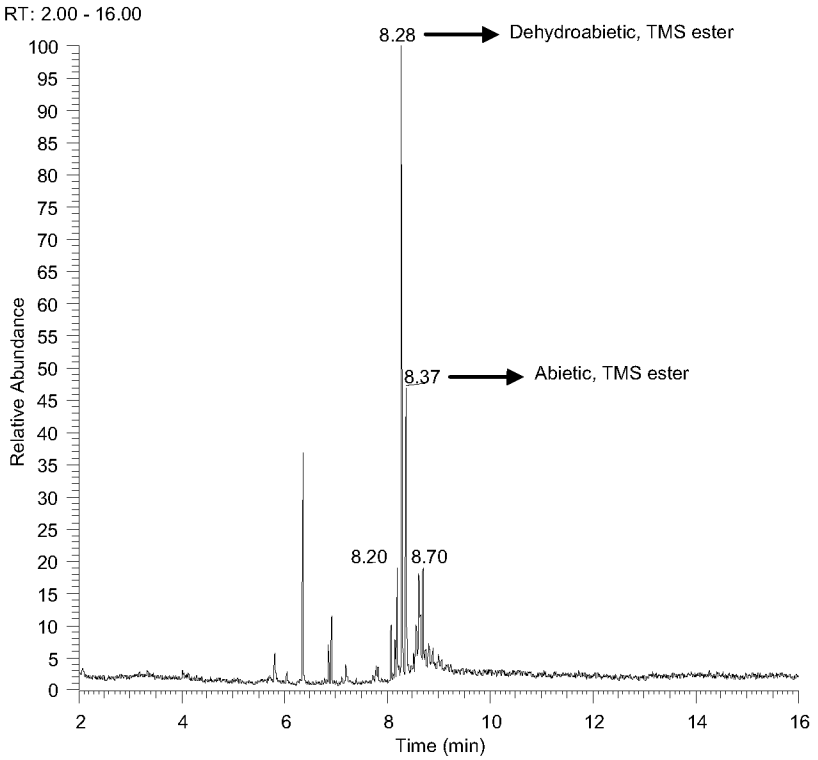

FIG 2. Presence of nonproteic, low-molecular-weight substances in the extract was analyzed by using gas chromatography combined with mass spectrometry. The extract mainly contains abietic acid (23%) and dehydroabietic acid (51%) among other nonidentified subtances. *RT,* Retention time; *TMS,* trimethylsilyl.

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Opposite effects of allergy prevention depending on *CD14* rs2569190 genotype in 3 intervention studies

Several birth cohort studies aimed at preventing the development of asthma by reducing the amount of exposure to inhalant allergens found no effects or even opposite effects on atopy development.[1-3] The Dutch Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study investigated whether the use of mite allergen–impermeable mattress covers (IMCs) reduced the risk of asthma and allergy in high-risk children. No effect was found, despite lower allergen levels on the children's mattresses.[1] The Dutch Prevention of Asthma in Children (PREVASC) study and Canadian Childhood Asthma Primary Prevention Study (CAPPS) assessed the effectiveness of a multifaceted intervention program for primary prevention of asthma in high-risk children, including the use of mattress covers. A meta-analysis of the PREVASC study, CAPPS, and the Isle of Wight study showed a decreased risk of asthma with multifaceted interventions in the first 5 years of life.[4] Although this effect was still observed in CAPPS at 7 years of age, the prevalence of atopy was not reduced.[2] A possible explanation might be that the interventions simultaneously reduced microbial exposure in the children's mattresses, which are important reservoirs of bacteria and microbial products.[5] This would support the hypothesis that binding of microbial antigens to innate immune receptors skews the immune system toward a T_H1 response, favoring a nonallergic immune response. A common functional single nucleotide polymorphism in the innate receptor *CD14* (rs2569190) has been shown to interact with the level of microbial exposure in the development of atopy.[6-8] Therefore we studied whether the rs2569190 genotype modifies the intervention effects on the development of asthma and allergy in the PIAMA, CAPPS, and PREVASC birth cohorts.

We compared distributions of doctor-diagnosed asthma and symptoms of asthma (wheeze, dyspnea, or use of inhaled corticosteroids) in the last 12 months, atopy (≥1 positive skin prick test

PREPARATION OF EXTRACTS

Wood dust was extracted with PBS buffer (0.1 mol/L sodium phosphate, pH 7.0, and 0.15 mol/L NaCl; 1× 1:5 [w/v], 1 hour, 4°C) and centrifuged (10,000×*g*, 30 minutes, 4°C). The supernatant was dialyzed (cutoff point, 3.5 kDa) against H_2O and freeze-dried. The protein concentration was quantified according to the method of Bradford (Pierce Biotechnology, Inc, Rockford, Ill).

ELISA INHIBITION AND CROSS-INHIBITION IMMUNOASSAYS

ELISA cross-inhibition studies were performed according to established methods.[E1] The 96-well polystyrene plates (Costar) were coated with 30 μg/mL of each wood extract to which the patient showed positive specific IgE (obeche, pine, and iroko). Patient serum was preincubated with obeche, oak, pine, cerejeira, and iroko at final concentrations of 100, 10, 1, and 0.1 μg/mL at room temperature for 3 hours. Subsequently, the inhibitor mixtures (including serum with no inhibitor as positive control) were added to the obeche-, pine-, and iroko-coated plates and incubated at 37 °C for 1 hour. The assay was completed by incubating with rabbit anti-human IgE antibody (1:3000; DAKO A/S, Glostrup, Denmark). IgE binding was detected by using *o*-phenylenediamine tablets (OPD, DAKO), and the reaction was stopped by adding 2 mol/L of chloric acid. The absorbance (optical density [OD]) in each well was measured at 490 nm. For inhibition ELISA with pine and abietic acid, the plate was coated with 30 μg/mL of pine extract and patient serum was incubated with pine and abietic acid at final concentrations of 1000, 100, 10, 1, and 0.1 μg/mL.

The percentage of inhibition of IgE binding was calculated by using the following formula:

$$\% \text{ Inhibition} = 100 - [\text{OD inhibited} - \text{OD blocking}/\text{OD noninhibited} - \text{OD blocking} \times 100].$$

WESTERN-BLOT ASSAY WITH OBECHE AND PINE EXTRACTS

Obeche and pine wood dust extracts (30 μg) were separated on SDS-PAGE.[E2] Proteins were electrotransferred to polyvinylidene difluoride membranes. Blocked membranes (Blocking solution, Sigma, St Louis, Mo) were incubated with patient serum (1:10 dilution). The IgE binding was revealed with anti-human IgE-peroxidase conjugate (Biosource, Camarillo, Calif; 1:3000 dilution) and chemiluminiscence (Amersham Supersignal West Pico Chemiluminutoescent Substrate, Rockford, Ill).

GAS CHROMATOGRAPHY–MASS SPECTROMETRY ANALYSIS

The chromatograms were registered by using a Finnigan Trace GC Ultra (Thermo Electron Corporation, Waltham, Mass) coupled with a Trace DSQ Quadrupole MS (Thermo Electron Corporation) and an autoinjector AI3000. The gas chromatograph was equipped with a capillary column coated with 5% phenyl-95% dimethylpolysiloxane: DB-5 (15 m × 0.25 mm, 0.25-μm film thickness). A standard of abietic acid (Sigma-Aldrich) was run in the gas chromatograph combined with mass spectrometer for the identification of the peak. Both samples (10 mg) (reference sample of abietic acid and our pine extract) were dissolved in dry pyridine (1 mL) and derivatized with the addition of *N,O*-bis(trimethylsilyl)trifluoroacetamide (Sigma-Aldrich) (0.7 mL) for 30 minutes before the analysis. Injection volume was 1 μL. Splitless injection (the splitless time was set to 1 minute) of extracts and a split ratio of 50:1 of the reference was performed with the autosampler into an injector at 250°C. The gas chromatograph column temperature was programmed as follows: 80°C isothermal for 2 minutes, 30°C/min from 80 to 280°C, and 300°C isothermal for 7.5 minutes. The transfer line was held at 250°C. The mass spectrometry was operated in electron ionization mode at 70 eV, and the mass range was from *m/z* 40 to 500. In the resulting chromatogram, the peak at 8.37 minutes retention time corresponds to derivative compound of abietic acid and the one at 8.28 corresponds to the dehydroabietic acid one. The other abundant peak at a retention time of 6.37 minutes and other less abundant as those at 6.86 and 6.92 minutes should not be taken into account since they correspond to by-products of the derivatizing reagent. The identification of the abietic acid trimethylsilyl ester was done by running the reference sample (retention time was 8.37 minutes). In addition, computer matching of the mass spectra from the NYST library was consistent. In the case of dehydrabietic acid trimethylester, the identification was confirmed by using computer matching.

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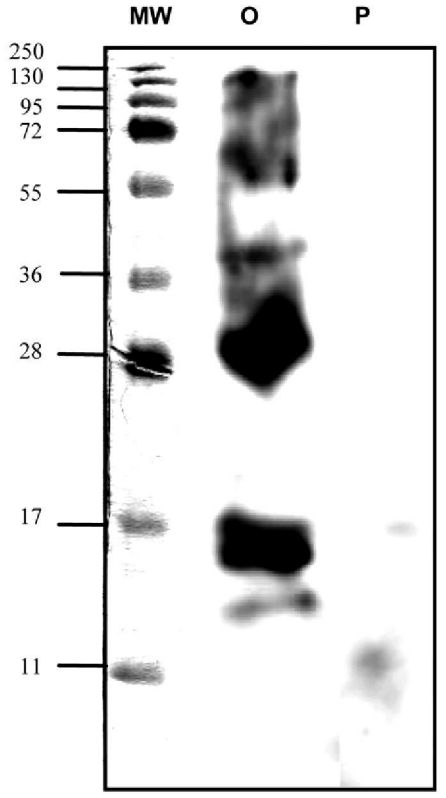

FIG E1. Western blot performed with the patient's serum and obeche *(O)* and pine tree *(P)* extracts. Patient's serum recognized bands of approximately 14, 28, 38, and 58 kDa in the obeche extract but none in the pine extract. *MW,* Molecular weight.